Modi-2, a vaccine targeting homocitrullinated self-epitopes, stimulates potent CD4-mediated anti-tumour responses as a therapy for solid cancers

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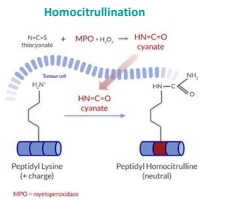
INTRODUCTION

- The tumour microenvironment (TME) is subject to stressful conditions such as nutrient deprivation, genotoxic stress and hypoxia which force cancer cells to undergo autophagy where cellular proteins are targeted for degradation.
- Stresses within the TME also mediate post-translational modification of self-proteins.
- Post-translational modification can generate neoepitopes and bypass self-tolerance.[1-3]
- Homocitrullination (Hcit) or carbamylation is the conversion of lysine to homocitrulline which can be mediated by myeloperoxidase enzyme (MPO) produced by neutrophils, macrophages and MDSCs in the TME.[3,4]
- Here we describe a vaccine comprising of 4 Hcit peptides, homologous in mice and humans, that mediates tumour therapy in murine models.



Modi-2 vaccine stimulates Hcit specific CD4 responses *in vivo*

- Studies were carried out using transgenic mice expressing different human HLA types or wild type Balb/c mice.
- Mice were immunised with Hcit peptides with adjuvant CpG/MPLA.
- Peptide specific responses were assessed by IFNγ ELISpot.
- Specific CD4 responses were observed to the Hcit peptides.

Table 1. Peptides in Modi-2 vaccine and HLA restrictions

	DR1	DR4	DP4	Balb/c (I-Ad/I-Ed)
Vim 116-135^{Hcit}	✓	✓	×	✓
Aldo 74-93^{Hcit}	×	×	✓	✓
BiP 562-579^{Hcit}	×	ND	✓	×
Cyk8 371-388^{Hcit}	✓	ND	✓	✓

ND = not determined

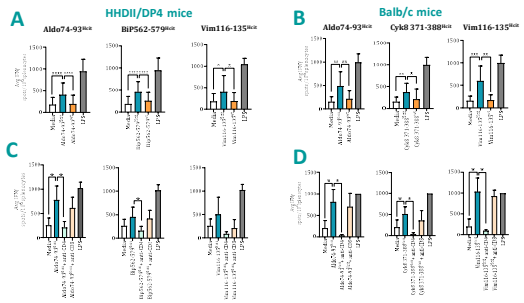


Figure 1. HHDII (HLA-A2)/DP4 transgenic (A & C) or Balb/c (B & D) mice immunised with combination Hcit peptide vaccine (Modi-2) with CpG and MPLA adjuvants on days 1, 8 and 15 and peptide specific responses assessed by *ex vivo* IFNγ ELISpot assay on day 21 to Hcit or native peptides (A & B) or to Hcit peptides in the presence of CD4 or CD8 blocking antibodies (C & D) (n>5). LPS stimulus used as a positive control and media only as negative control. **** p<0.0001, *** p<0.0005 ** p<0.005 * p<0.05 by ANOVA.

Modi-2 vaccine provides tumour therapy in mouse tumour models

- Studies were carried out using HHDII/DP4 transgenic mice or wild type Balb/c mice.
- Immunisation with Hcit peptides with CpG/MPLA adjuvant following B16 HHDII/iDP4 or CT26 tumour mediates tumour therapy.
- Tumour free survivors in CT26 model were rechallenged with tumour at day 50 with no additional immunisation and showed protection against rechallenge.

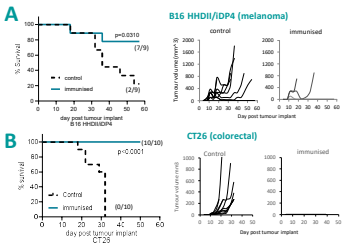


Figure 2. HHDII/DP4 transgenic (A) or Balb/c (B) mice were injected with 1x10^5 B16 HHDII/DP4 or 1.75x10^6 CT26 tumour respectively on day 1 followed by immunisation with Hcit peptides in CpG/MPLA adjuvants on days 4, 11 and 18 or days 4, 8 and 11. Overall survival and tumour growth monitored. Numbers in brackets represent the number of tumour free mice out of the total at day 50. (C) tumour free survivors from CT26 model were rechallenged with CT26 tumour at day 50 without further immunisation and survival compared to a new unimmunised control group. Survival analysed using LogRank test.

Humans have a repertoire of T cells responding to Hcit peptides and human tumours express Modi-2 target antigens

- PBMCs were isolated from healthy donors and stimulated with each peptide for 8-10 days.
- Peptide specific restimulation responses were measured by IFNγ ELISpot.
- Healthy donors show IFNγ responses to Hcit peptides.

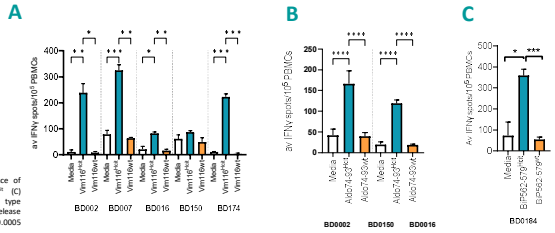


Figure 3. PBMC cultures grown in the presence of Vim116-135^{Hcit} (A), Aldo74-93^{Hcit}, BiP562-579^{Hcit} (C) peptides were restimulated with Hcit or wild type peptides and media (negative control). IFNγ release measured by ELISpot assay. **** p<0.0001, *** p<0.0005 ** p<0.005 * p<0.05 by ANOVA.

- Human tumour samples stained for antigen and expression and homocitrullination by immunohistochemistry.
- All tumours show expression of 4 antigens, which is increased in some cancer tissue compared to normal or normal adjacent tissue.
- All tumours show evidence of homocitrullination.

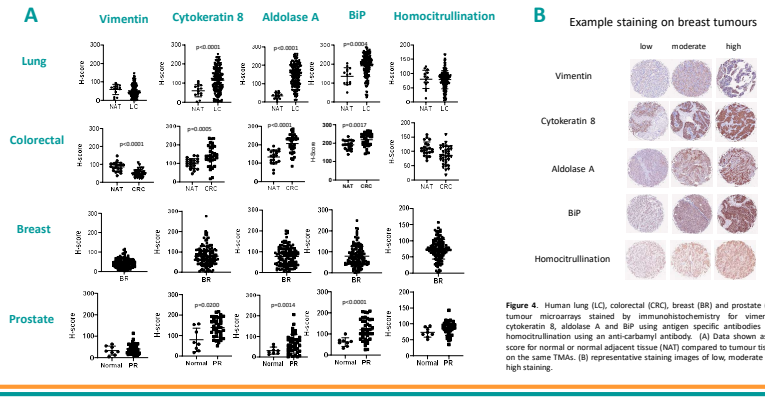


Figure 4. Human lung (LC), colorectal (CRC), breast (BR) and prostate (PR) tumour microarrays stained by immunohistochemistry for vimentin, cytokeratin 8, aldolase A and BiP using antigen specific antibodies and homocitrullination using an anti-carbamyl antibody. (A) Data shown as H-score for normal or normal adjacent tissue (NAT) compared to tumour tissue on the same TMAs. (B) representative staining images of low, moderate and high staining.

Modi-2 vaccine as a SNAPvax formulation stimulates immune responses and mediates tumour therapy in mice

- SNAPvax formulation links TLR7/8 adjuvant to peptide to ensure co-delivery to APCs.[5-7]
- Improved GMP manufacturing and uniform compositions.

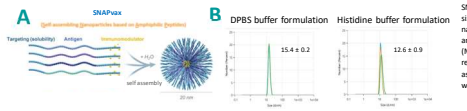


Figure 5. (A) Schematic of SNAPvax technology, (B) Particle size distribution profile of mosaic nanoparticles analysed by multi-angle dynamic light scattering (MADLS) using a Zetasizer ultra red instrument. Data presented as mean particle size (nm ± SD) with distribution graph.

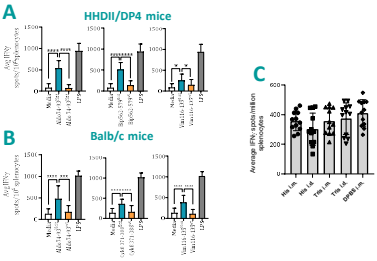


Figure 6. HHDII/DP4 transgenic (A) or Balb/c (B) mice immunised with 4-10nmol dose Modi-2 SNAPvax on days 1, 8 and 15 and peptide specific responses assessed by ex vivo IFNγ ELISpot assay on day 21 to Hcit or native peptides (n>5). LPS stimulus used as a positive control and media only as negative control. **** p<0.0001, *** p<0.0005 ** p<0.005 * p<0.05 by ANOVA. (C) responses to pooled Modi-2 peptides in Balb/c mice immunised with 10nmol Modi-2 SNAPvax in Histidine, Tris or DPBS buffers via intradermal (i.d.) or intramuscular (i.m.) routes. Values normalised against media control.

- HHDII/DP4 and Balb/c mice were immunised with Modi-2 SNAPvax formulation.
- Modi-2 SNAPvax formulations stimulates similar responses in HHDII/DP4 and Balb/c mice.
- Delivery of Modi-2 formulation in Histidine, Tris or DPBS buffers via intradermal (i.d.) or intramuscular (i.m.) routes generates similar responses.

- Studies were carried out using HHDII/DP4 transgenic mice or wild type Balb/c mice.
- Immunisation with Modi-2 SNAPvax following B16 HHDII/iDP4, 4T1 or CT26 tumour.
- Modi-2 SNAPvax mediates tumour therapy in several tumour models.
- Modi-2 SNAPvax immunised mice show evidence of increased T cell infiltrate into tumours and increased MHCII expression.

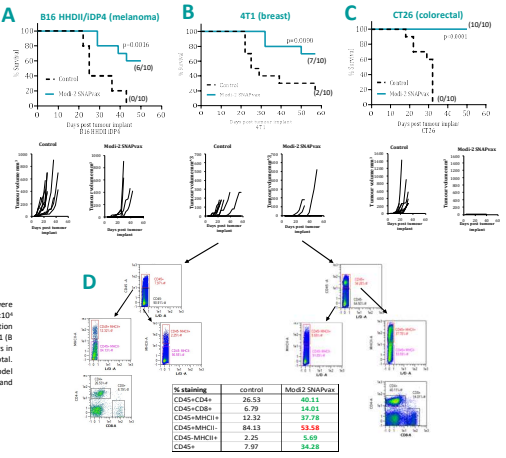


Figure 7. HHDII/DP4 transgenic (A) or Balb/c (B and C) mice were injected with 1x10^5 B16 HHDII/DP4 (A), 5x10^5 4T1 (B) or 1.75x10^6 CT26 (C) tumour respectively on day 1 followed by immunisation with Modi-2 SNAPvax on days 4, 11 and 18 (A) or days 4, 8 and 11 (B and C). Overall survival and tumour growth monitored. Numbers in brackets represent the number of tumour free mice out of the total. Survival analysed using LogRank test. (D), Tumours from 4T1 model disaggregated and stained *ex vivo* for CD45, CD8, CD4 infiltrate and MHCII expression. Representative tumour examples shown.

CONCLUSIONS

- **Hcit peptides stimulate CD4 T cell responses in standard and HLA transgenic mice that mediate tumour therapy.**
- **Humans have a repertoire of T cells that are specific to Hcit peptides.**
- **Human tumours show evidence of homocitrullination and express the self-antigens from which Modi-2 vaccine peptides are derived.**
- **Modi-2 peptides can be formulated with SNAPvax technology enabling improved solubility and GMP manufacture and Modi-2 SNAPvax mediates strong immune responses and tumour therapy in mouse models.**

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